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Original Contribution

OXIDATIVE DAMAGE IN THE GUINEA PIG HIPPOCAMPAL SLICE

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Abstract—Free radicals and active oxygen compounds are implicated in brain ischemia and head trauma. Previous studies have shown that free radicals, generated by radiation and through the Fenton reaction, produce both synaptic and postsynaptic damage in the hippocampal brain slice.[1,2,3] To evaluate the contribution of oxidation to the observed damage, the actions of the oxidants, chloramine-T and N-chlorosuccinimide (NCS), were studied on electrophysiological responses in the hippocampal slice isolated from the brains of guinea pigs. Electrical stimulation of afferents to neurons of the CA1 region of hippocampus evoked a population postsynaptic potential (population PSP) in the dendritic layer and a population spike in the cell body layer. Chloramine-T (25–500 μM) and NCS (750–4000 μM) decreased the population spike in a dose-dependent manner ($ED_{50} \approx 125$ μM and 1100 μM, respectively). Input/output curves revealed that both the population spike and the population PSP were significantly reduced with both oxidants; but, the ability of the population PSP to produce a population spike was not impaired. These studies suggest that oxidation reactions can account for the synaptic component of the damage produced by free radicals but can not account for the postsynaptic effects.

Keywords—Chloramine-T, N-chlorosuccinimide, Oxidation, Free radical, Hippocampus

INTRODUCTION

Active oxygen compounds are generated normally in vivo[4,5,6] but the presence of superoxide dismutase, catalase, peroxidase and a variety of antioxidants limit the concentrations to non-toxic levels. In the event of an ischemic attack or head trauma, levels of these active oxygen species increase,[7,8] however the source of these compounds is unclear. Peroxide and superoxide could be generated from xanthine oxidase in local endothelial cells[9,10]; Beckman et al.[9] calculated concentrations of superoxide and peroxide as high as 70 μM/min and 170 μM/min, respectively, following an ischemic episode. Active oxygen compounds could also be secreted by the microglia invading a region of injury.[11] Another possible source is the generation of free radicals in neurons during reperfusion when a burst of oxidative metabolism results in the release of incompletely reduced oxygen (i.e., superoxide and peroxide).[8]

Previous studies have shown that active oxygen species produce functional damage in neurons.[1,2,3] Hydrogen peroxide and ionizing radiation decrease synaptic efficacy (synpatic damage) and impair mechanisms of spike generation (postsynaptic damage). The evidence suggests that the molecular mechanisms underlying synaptic and postsynaptic damage are different.[3] In the present study, the oxidants chloramine-T and N-chlorosuccinimide were tested to evaluate the contribution of an oxidation reaction to free radical damage. The results indicate that the oxidants can account for impairment of synaptic function but not for postsynaptic deficits.

MATERIALS AND METHODS

Hippocampal slices were prepared from the brains of euthanized male Hartley guinea pigs as previously described.[1,2] The slices (400–450 μm thick) were incubated in artificial cerebrospinal fluid (ACSF) (composition in mM: 124 NaCl, 3 KCl, 2.4 $CaCl_2$, 1.3 $MgSO_4$, 1.24 KH_2PO_4, 10 glucose, 26 $NaHCO_3$, equilibrated with 95% O_2/5% CO_2) at room temperature for 1–2 hours to allow recovery from dissection. A slice was then transferred to a submerged slice recording chamber and continually perfused (1–2 ml/min) with oxygenated ACSF. All experiments were done at 30° ± 1° C. Solutions of chloramine-T (CT) and N-

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chlorosuccinimide (NCS) (Sigma Chemical Company) were prepared fresh daily.

Potentials were recorded with a high gain DC amplifier and were digitized, stored, and analyzed on an LSI 11–23 minicomputer. A bipolar stainless steel stimulating electrode (DKI) was positioned in stratum radiatum to activate the Schaffer collateral pathway as well as other afferents to the CA1 pyramidal cells. Constant current stimuli (0.1–1 mA, 200 μs) were provided at 0.20 Hz. Field potentials were recorded using glass microelectrodes filled with 2M NaCl and having a resistance of less than 10 MΩ. One recording electrode was placed in the cell body layer of CA1 region to record the somatic response (population spike). The population spike is the extracellularly recorded action potential occurring synchronously in a population of CA1 pyramidal cells. In some experiments, a second recording electrode was positioned in the stratum radiatum to record the dendritic response (population postsynaptic potential, population PSP) and the afferent volley. The population PSP is the extracellularly recorded synaptic potential activated synchronously in the dendrites of CA1 pyramidal cells. The afferent volley is the potential produced by the activation of fibers in the stimulated pathway.

Following placement of the electrodes, baseline recordings were obtained for a minimum of 30 min to ensure stability of the tissue. Stimulus intensity was set to a value that produced approximately a half-maximal response. If during this period the responses changed substantially, the experiment was discarded. A dose of either chloramine-T or NCS was then applied

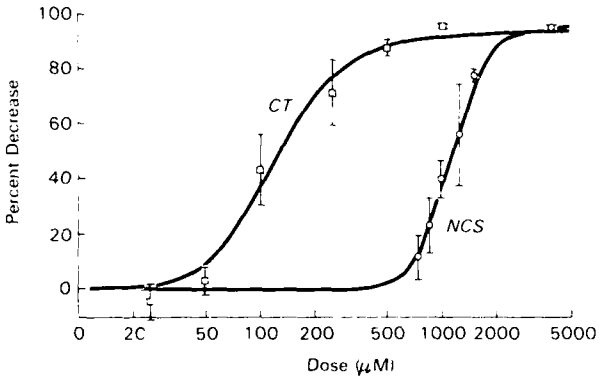

Fig. 1. Dose response curves for Chloramine-T (CT) and NCS. Varying doses of chloramine-T and NCS were applied to the hippocampal brain slice for 30 min. The percent decrease in the population spike at 30 min in comparison with control (i.e., before drug) is plotted vs. the dose of drug used. The number of experiments with Chloramine-T at 25 μM = 6; 50 μM = 7; 100 μM = 6; 250 μM = 7; 500 μM = 5; 1000 μM = 3. The number of experiments with NCS at 625 μM = 5; 750 μM = 4; 875 μM = 6; 1000 μM = 7; 1250 μM = 5; 1500 μM = 4; 4000 μM = 8. Squares: Chloramine-T; Circles: NCS.

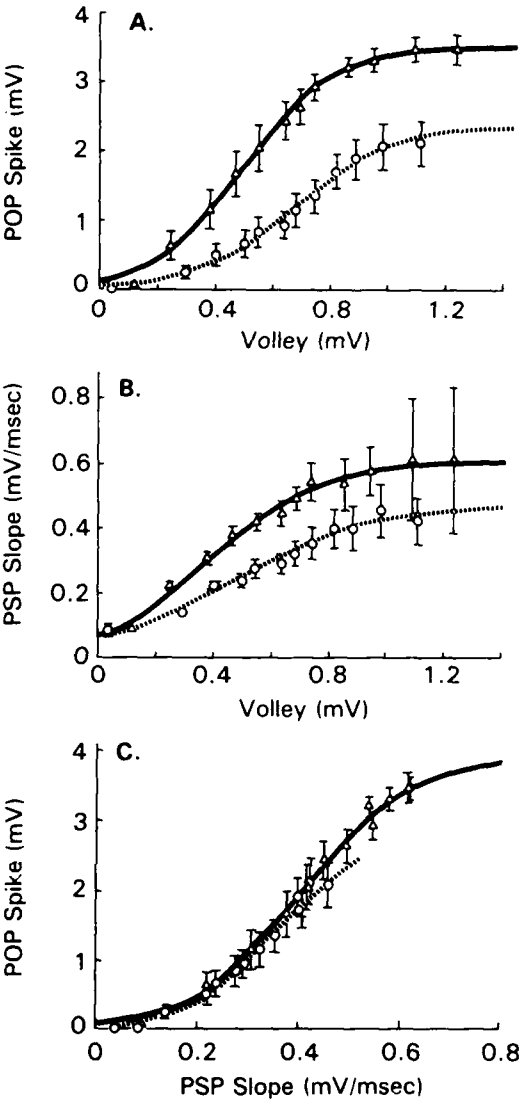

Fig. 2. Input-output curves averaged for 8 slices exposed to 100 μM chloramine-T. Control curves: solid line; chloramine-T curves: dotted line. A: Graph shows that chloramine-T reduces the population spike for a given afferent volley amplitude. B: Chloramine-T decreases the ability of the afferent volley to produce a synaptic response. C: Chloramine-T has no affect on the ability of a PSP to evoke an action potential. Chloramine-T causes synaptic but no postsynaptic deficits.

continuously for thirty minutes. Electrophysiological responses were continuously monitored throughout this period; every 5 min 8 traces were averaged. Drug was then washed off and the tissue was perfused with ACSF for another 30 min. To determine the dose-response relationships, a minimum of four experiments at each dose of NCS and chloramine-T were performed. The effectiveness of the drug was expressed as the percentage decrease in amplitude at the 30 min time point as compared to control amplitude. The 30 min time point was chosen for 2 reasons: 1) The drug effects

frequently appeared to level off within this time period and 2) this protocol allowed comparison with previous experiments on hydrogen peroxide using a 30 min exposure. All experiments at a single dose were averaged and standard errors (SEM) were calculated.

Input-output (I/O) curves were constructed for each drug at a dose that produced approximately a 40% decrease in population spike amplitude. Somatic and dendritic traces ($n = 4$) were averaged at each stimulus intensity ranging from 0.1 to 1.0 mA. The data from 5 experiments for NCS and for 8 experiments for chloramine-T were averaged to obtain composite curves. The I/O curves consisted of three relationships: 1) volley vs. population spike 2) volley vs. population PSP, and 3) population PSP vs. population spike. The volley vs. population spike curve reflects the ability of the presynaptic activity to elicit an action potential in the postsynaptic cell. The population spike vs. population PSP reflects primarily postsynaptic mechanisms (i.e., the effectiveness of a postsynaptic depolarization to evoke an action potential). The volley vs. population PSP reflects the synaptic mechanisms (i.e., the ability of presynaptic activity to produce a synaptic potential). Thus, analysis of these curves provides information on the mechanisms of oxidative damage. The input/output data were computer-fitted with the equation for a sigmoid curve and analyzed as previously described.[3] Differences between control and treated curves were tested for significance by comparing the residual sum of squares for the individual curves with the residual sum of squares for a curve fit to a composite of control and test data. Significance was accepted at $p < 0.05$.

RESULTS

Chloramine-T decreased the amplitude of the population spike elicited by orthodromic stimulation of CA1 in a dose dependent manner (Fig. 1). Low doses of chloramine-T (25 μM) produced little or no effect during or after exposure to the drug. Intermediate doses (50–200 μM) noticeably decreased the amplitude of the population spike during the 30 minute exposure to chloramine-T. The response recovered approximately to its original size within a 30 min period of wash with normal ACSF. When exposed to higher doses of chloramine-T (250–1000 μM), the amplitude of the population spike decreased sharply during exposure and remained depressed throughout the wash. The dose response curve (Fig. 1) shows that the concentration for a half maximal effect is 125 μM. Chloramine-T is maximally effective at doses greater than about 500 μM.

The dose response curve for N-chlorosuccinimide (NCS) (Fig. 1) shows that NCS required higher concentrations than chloramine-T to produce comparable damage. The onset and reversal of NCS action was very similar to, although slightly more gradual than, that of chloramine-T. The dose of NCS to produce a half maximal effect was approximately 1.1 mM. Doses of 2 mM and greater caused a maximal decrease in the population spike amplitude.

Input-output (I/O) experiments were done to determine the site of damage of the oxidants. The dose of chloramine-T chosen for these experiments (100 μM) was expected from the dose response curve to produce, on the average, a 40% decrease in the population spike. The I/O curves ($n = 8$) show that for a given afferent volley input, both the population spike (Fig. 2A) and the popultion PSP (Fig. 2B) were significantly reduced by chloramine-T. The ability of the population PSP to produce a population spike, however, was not altered (Fig. 2C). The population PSP was equally effective in evoking a spike before and during exposure to the oxidant. Fig. 3 shows sample traces from a typical slice

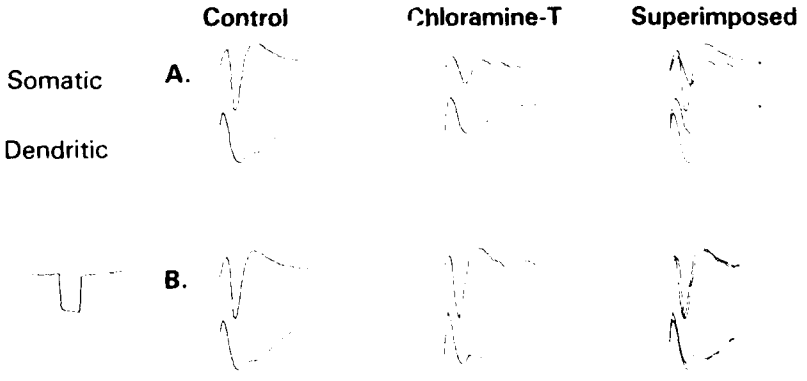

Fig. 3. Somatic (top traces) and dendritic responses (bottom traces) from slice treated with 100 μM chloramine-T. Control (left) and treated (middle) traces are superimposed on the right. A: At the same stimulus strength, Chloramine-T causes a decrease in both the population spike and the population PSP. B: When the stimulus strength in chloramine-T was increased to produce the same dendritic response as control, the evoked population spike was the same as control. Calibration (square wave on lower left): 1 mV (somatic), 0.5 mV (dendritic); 2 ms.

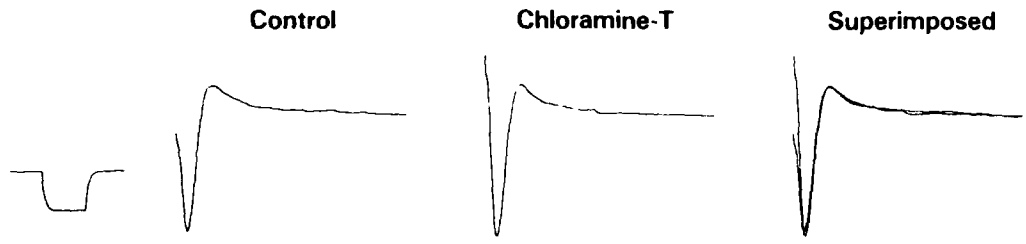

Fig. 4. The antidromic spike was evoked by stimulation of the alveus and recorded in the cell body layer of CA1. It was unaffected by 250 μM chloramine-T (center trace) and can be seen best when the traces are superimposed (right traces). Calibration (square wave on lower left): 1 mV, 2 ms.

treated with 100 μM chloramine-T. Both population spike and population PSP were reduced. If, while still in chloramine-T, stimulus strength was increased to produce a population PSP comparable to control, the evoked population spike is comparable in amplitude (Fig. 3B). These data suggest that the observed decrease in the population spike with exposure to chloramine-T is due completely to the reduction in the synaptic response.

Previous reports[12,13,14,15,16] indicate that chloramine-T at similar doses removes sodium inactivation, broadening the sodium action potential in squid, crayfish, frog and toad axons. To evaluate the actions of chloramine-T directly on the sodium action potential in this preparation, the effect on the antidromically elicited population spike was evaluated. Axons of the CA1 pyramidal cells were stimulated with a bipolar stainless steel electrode in the alveus. The resulting antidromic spike was recorded from the cell body layer of CA1. At a dose that maximally reduced the synaptically-activated (orthodromic) population spike (250 μM), chloramine-T had no effect on the antidromic action potential (Fig. 4) ($n = 4$).

Input/output experiments were repeated with NCS at a dose of 1.0 mM ($n = 5$). As with chloramine-T, the population spike (Fig. 5A) and the population PSP (Fig. 5B) evoked by equivalent afferent volleys were both reduced. Yet population PSPs of equivalent size, before and during exposure to NCS, produced equivalent population spikes (Fig. 5C). NCS reduced the population PSP for the same size afferent volley, but the effectiveness of that PSP was unaltered. As with chloramine-T, these results demonstrate that only synaptic damage and no postsynaptic deficits were produced by NCS.

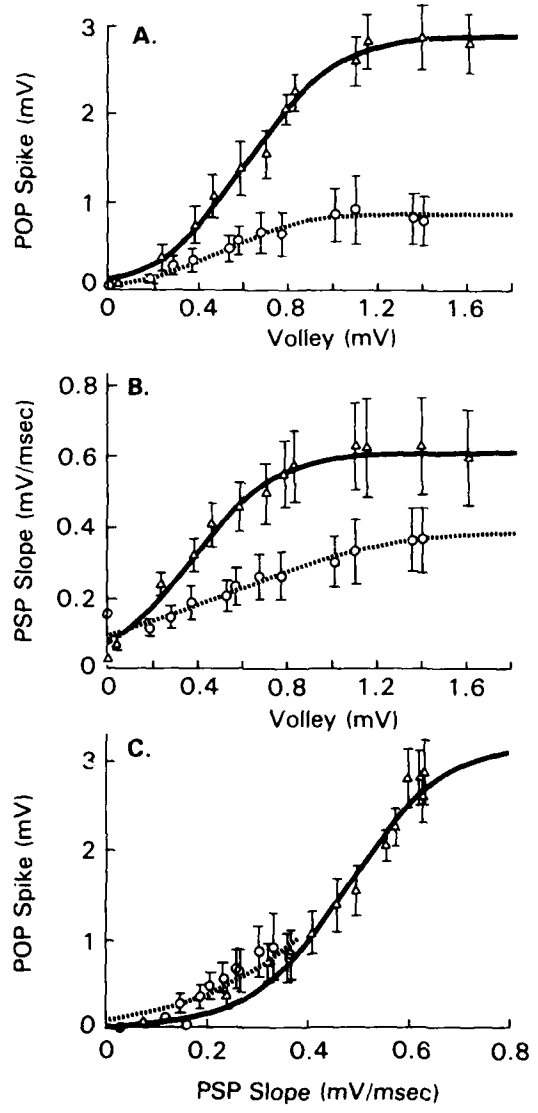

Fig. 5. Input-output curves averaged for 8 slices exposed to 1 mM NCS. The results are identical to those obtained for chloramine-T. Control curves: solid line; NCS curves: dotted line. A: For a given afferent volley, the population spike is decreased. B: For a given afferent volley, the synaptic response is decreased. C: For a given synaptic response, the evoked population spike is the same both in control and in NCS. NCS causes only synaptic damage and no postsynaptic damage.

DISCUSSION

The oxidants, chloramine-T and N-chlorosuccinimide, were observed to cause a decrease in the orthodromic potentials in the CA1 region of the hippocampus in vitro. While the synaptic potentials were reduced

in size, they were not impaired in their ability to evoke action potentials in the postsynaptic neurons. The reduced synaptic efficacy occurred at doses that did not alter the antidromically-evoked spike in the same neuronal population.

Chloramine-T and NCS have been reported to remove inactivation of the sodium current in the giant axon of squid[14,16] and crayfish[16] and in the myelinated axons of the toad[12,13] and frog.[15] Although many of the studies used chloramine-T in higher doses (1 to 10 mM), Wang[12] reported that in toad fibers, a dose as low as 89 μM was effective in broadening the action potential 3-fold after only 7 min. Longer exposures further prolonged the action potential and began to reduce the amplitude. In contrast, in the present study in the hippocampal slice preparation 250 μM chloramine-T for 30 min did not alter the antidromic spike which is predominantly a sodium-dependent action potential. One major difference between the present experiments and those testing chloramine-T in axons is that in the present study, potassium currents were not pharmacologically blocked. The presence of the potassium component of the action potential could obscure an action of the oxidants to broaden the action potential through removal of sodium inactivation. In two studies[12,14] chloramine-T (1–14 mM) was also found to decrease the potassium current in toad and squid axons. Neither decreased sodium inactivation or decreased potassium current can account for the decrease in synaptic efficacy seen in the present study. If anything, one would predict that both mechanisms would increase the duration of the action potential, increase the presynaptic calcium influx and enhance transmitter release.

Previous studies[1,2,3] showed that ionizing radiation and hydrogen peroxide could produce damage in an isolated neuronal system. In an aqueous environment ionizing radiation produces free radicals including ·OH and O_2^-. Hydrogen peroxide can react with iron and through the Fenton reaction produce hydroxyl free radicals. Both procedures decreased synaptic responses produced by orthodromic stimulation of stratum radiatum suggesting either a decrement in presynaptic release or impaired function of the postsynaptic receptor/ionophore complex (synaptic damage). In addition, the synaptic potentials that were elicited were less capable of evoking an action potential in the hippocampal neurons suggesting that membrane properties of the soma or axon hillock were altered in some way (postsynaptic damage). Two separate mechanisms were postulated for these two actions since they showed very different dependencies on the dose rate of radiation.[3] Since free radicals produced by ionizing radiation (such as the hydroxyl free radical) and hydrogen peroxide[17] are oxidants, the contribution of an oxidative reaction to the observed damage was considered.

Chloramine-T and NCS are oxidizing agents that appear to be fairly specific for methionine and cysteine residues of membrane proteins.[18] These actions are similar to the oxidizing effects of hydrogen peroxide.[17] The oxidants in the present study were able to produce damage in the hippocampal brain slice, but they could not completely mimic the effects of radiation and peroxide. This provides further support for the hypothesis that two separate mechanisms underlie the synaptic and the postsynaptic damage produced by radiation and by peroxide. The present study showed that the oxidants specifically impaired synaptic efficacy, suggesting that an oxidation reaction could account for the peroxidative and radiation damage at this site. Yet, the oxidants had no effect on postsynaptic generation of action potentials. The limited dose rate dependence of radiation damage[3] and the iron potentiation of peroxide damage[1,2] are also more consistent with a lipid peroxidation mechanism than with an oxidation reaction.

In conclusion, the observations reported here suggest that oxidative damage may account for the synaptic component of free radical damage in the nervous system but can not account for the postsynaptic deficits.

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